



Draft Genome Sequence of the Biofuel-Relevant Microalga Desmodesmus armatus

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ABSTRACT A draft genome of 906 scaffolds of 115.8 Mb was assembled for *Desmodesmus armatus*, a diploid, lipid- and storage carbohydrate-accumulating microalga proven relevant for large-scale, outdoor cultivation, and serves as a model alga platform for improving photosynthetic efficiency and carbon assimilation for next-generation bioenergy production.

icroalgae, having higher annual biomass production relative to terrestrial crops and potential for cultivation in salt water on nonarable lands, are critical future feedstocks for renewable liquid fuels (1-4). Desmodesmus, taxonomically divergent from the genus Scenedesmus (5, 6), has been shown to exhibit phenotypic plasticity and resilience, allowing survival in harsh and variable environments, including brackish and salt water (7–12), and to possess other beneficial traits for outdoor, large-scale, biofuelrelevant cultivation, as follows: it thrives in temperatures of 35 to 45°C (13-15), resists toxins (12) and grazers (16), accumulates microcrystalline guanine and polyphosphate (17) and lutein as an important coproduct for aquaculture (15), settles rapidly (13), and has diverse, sporopollenin-containing cell walls (18-20). Desmodesmus achieved areal harvest yield productivities of 9 to 20 g/m² day (annual average of 11.4 g/m² day) over the course of 2 years in outdoor, large-scale saltwater cultivation (2, 11, 21, 22) and can accumulate >55% dry cell weight (dcw) lipids (14, 23) or carbohydrates (13), both important cellular components for biofuels, making it amenable to a fractionationbased conversion pathway (24-26). Here, we report the draft nuclear and chloroplast genomes, ploidy, and potential bioenergy-relevant engineering targets of D. armatus.

D. armatus (Fig. 1), originally isolated from Las Cruces, NM, wastewater treatment ponds in 2012, was grown photoautotrophically in flat-sided bottles in modified artificial seawater medium (MASM) under constant illumination (4000K LED flat panels, 180 μ mol photons/m²), supplemented with 2% CO₂. MASM contains the following (g/liter): NaCl (8), MgSO₄·7H₂O (2.5), KCl (0.6), NaNO₃ (0.85), CaCl₂·2H₂O (0.3), Tris (1), KH₂PO₄ (0.05), NH₄Cl (0.03), thiamine-HCl (3.5 mM stock; 1 ml/liter), cyanocobalamin $(10 \ \mu g/L \text{ stock}; 1 \text{ ml})$, and trace element stock (6 ml/liter). Trace element stock was made up of the following (g/liter): Na₂-EDTA (1.0), FeCl₃·6H₂O (0.2), MnCl₂·4H₂O (0.072), $ZnCl_2$ (0.02), Na_2MoO_4 ·2H₂O (0.013), and $CoCl_2$ ·6H₂O (0.004). Genomic DNA was extracted from midexponential cells as described (27). PacBio sequence data consisted of average polymerase reads of 741,936 \pm 76,024 bp, having a mean insert length of $6,026 \pm 784$ bp and generating a total assembly size of 116.31 Mb in 906 contigs with an N_{50} value of 341,806 bp and a GC content of 56.6%, and was determined to be diploid (28). This compares with the genomes of Tetradesmus obliquus (108.72 Mb; 29) and Scenedesmus obliquus (207.97 Mb; 30). Sixteen scaffolds (totaling 112,885 bp) contain chloroplast sequences identified using BLASTN against published chloroplast genomes for Chlorella vulgaris C-27 (31) and Monoraphidium neglectum (32). Proteins that may be beneficial for a robust, outdoor, bioenergy-relevant alga were identified.

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FIG 1 Typical cenobium with terminal spines and intracellular changes in morphology of *D. armatus* under nitrogen-replete and -deplete (inset) conditions. Nitrogen-deplete conditions occur in late-stage cultures once nitrogen is depleted from the medium.

The presence of homologs of *Chlamydomonas reinhardtii* STT7 and LHCSR1/3 and the dioxygen reductases Flv3 and PTOX confirms the ability for state transition and nonphotochemical quenching processes involved in energy dissipation essential for maintaining photosynthetic electron transport chain integrity in high or fluctuating irradiances (33) and may be useful targets for the improvement of photosynthetic efficiency and harvest yields in open ponds. Polyphosphate kinase was also identified, suggesting polyphosphate production as an energy or phosphate reserve. In conclusion, the innate robustness and proven reliability of *D. armatus* in outdoor mass culture provides a robust yet flexible chassis for genetic engineering efforts, potentially leading to the commercial use of *D. armatus* as a bioenergy-relevant alga.

Data availability. This whole-genome shotgun project has been deposited at DDBJ/ENA/GenBank under the accession number VIIQ00000000.

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REFERENCES

- Davis R, Coleman A, Wigmosta M, Markham J, Kinchin C, Zhu Y, Jones S, Han J, Canter C, Li Q. 2017. 2017 Algae harmonization study: evaluating the potential for future algal biofuel costs, sustainability, and resource assessment from harmonized modeling. NREL/TP-5100–70715. NREL, Golden, CO.
- White RL, Ryan RA. 2015. Long-term cultivation of algae in open-raceway ponds: lessons from the field. Ind Biotechnol 11:213–220. https://doi .org/10.1089/ind.2015.0006.
- 3. Knoshaug EP, Darzins A. 2011. Algal biofuels: the process. Chem Eng Prog 107:37–41.

- Williams PJIB, Laurens LML. 2010. Microalgae as biodiesel & biomass feedstocks: review & analysis of the biochemistry, energetics & economics. Energy Environ Sci 3:554–590. https://doi.org/10.1039/b924978h.
- Hegewald E. 2000. New combinations in the genus *Desmodesmus* (Chlorophyceae, Scenedesmaceae). Arch Hydrobiol Suppl Algol Stud 96:1–18. https://doi.org/10.1127/algol_stud/96/2000/1.
- Van Hannen E, FinkGodhe P, Lurling M. 2002. A revised secondary structure model for the internal transcribed spacer 2 of the green algae *Scenedesmus* and *Desmodesmus* and its implication for the phylogeny of these algae. Eur J Phycol 37:203–208. https://doi.org/10.1017/ S096702620200361X.
- Blokker P, Schouten S, van den Ende H, De Leeuw JW, Sinninghe Damsté JS. 1998. Cell wall-specific w-hydroxy fatty acids in some freshwater green microalgae. Phytochemisty 49:691–695. https://doi.org/10.1016/ S0031-9422(98)00229-5.
- Derenne S, Largeau C, Berkaloff C, Rousseau B, Wilhelm C, Hatcher PG. 1992. Non-hydrolysable macromolecular constituents from outer walls of *Chlorella fusca* and *Nanochlorum eucaryotum*. Phytochemisty 31: 1923–1929. https://doi.org/10.1016/0031-9422(92)80335-C.
- Gelin F, Volkman JK, Largeau C, Derenne S, Sinninghe Damsté JS, De Leeuw JW. 1999. Distribution of aliphatic, nonhydrolyzable biopolymers in marine microalgae. Org Geochem 30:147–159. https://doi.org/10 .1016/S0146-6380(98)00206-X.
- Gunnison D, Alexander M. 1975. Basis for the resistance of several algae to microbial decomposition. Appl Microbiol 29:729–738. https://doi.org/ 10.1128/AEM.29.6.729-738.1975.
- McGowen J, Knoshaug EP, Laurens LML, Dempster TA, Pienkos PT, Wolfrum E, Harmon VL. 2017. The Algae Testbed Public-Private Partnership (ATP³) framework; establishment of a national network of testbed sites to support sustainable algae production. Algal Res 25:168–177. https://doi.org/10.1016/j.algal.2017.05.017.
- Samori G, Samori C, Guerrini F, Pistocchi R. 2013. Growth and nitrogen removal capacity of *Desmodesmus communis* and of a natural microalgae consortium in a batch culture system in view of urban wastewater treatment: part I. Water Res 47:791–801. https://doi.org/10.1016/j.watres .2012.11.006.
- Ho S-H, Lai Y-Y, Chiang C-Y, Chen C-NN, Chang J-S. 2013. Selection of elite microalgae for biodiesel production in tropical conditions using a standardized platform. Bioresour Technol 147:135–142. https://doi.org/ 10.1016/j.biortech.2013.08.028.
- Pan Y-Y, Wan S-T, Chuang L-T, Chang Y-W, Chen C-NN. 2011. Isolation of thermo-tolerant and high lipid content green microalgae: oil accumulation is predominantly controlled by photosystem efficiency during stress treatments in *Desmodesmus*. Bioresour Technol 102:10510–10517. https://doi.org/10.1016/j.biortech.2011.08.091.
- Xie Y, Ho S-H, Chen C-NN, Chen C-Y, Ng I-S, Jing K-J, Chang J-S, Lu Y. 2013. Phototrophic cultivation of a thermo-tolerant *Desmodesmus* sp. for lutein production: effects of nitrate concentration, light intensity, and fed-batch operation. Bioresour Technol 144:435–444. https://doi.org/10 .1016/j.biortech.2013.06.064.
- Lurling M. 2003. Phenotypic plasticity in the green algae *Desmodesmus* and *Scenedesmus* with special reference to the induction of defensive morphology. Ann Limnol Int J Limnol 39:85–101. https://doi.org/10 .1051/limn/2003014.
- Moudrikova S, Nedbal L, Solovchenko A, Mojzes P. 2017. Raman microscopy shows that nitrogen-rich cellular inclusions in microalgae are microcrystalline guanine. Algal Res 23:216–222. https://doi.org/10.1016/ j.algal.2017.02.009.
- An SS, Friedl T, Hegewald E. 1999. Phylogenetic relationships of Scenedesmus and Scenedesmus-like coccoid green algae as inferred from ITS-2 rDNA sequence comparisons. Plant Biol 1:418–428. https://doi.org/ 10.1111/j.1438-8677.1999.tb00724.x.
- Trainor FR. 1998. Nova Hedwigia: biological aspects of Scenedesmus (Chlorophyceae) phenotypic plasticity, vol 117. J. Cramer, Berlin, Germany.

- Vanormelingen P, Hegewald E, Braband A, Kitschke M, Friedl T, Sabbe K, Vyverman W. 2007. The systematics of a small spineless *Desmodesmus* species, *D. costato-granulatus* (Sphaeropleales, Chlorophyceae), based on ITS2 rDNA sequence analysis and cell wall morphology. J Phycol 43:378–396. https://doi.org/10.1111/j.1529-8817.2007.00325.x.
- Knoshaug EP, Wolfrum E, Laurens LML, Harmon VL, Dempster TA, McGowen J. 2018. Unified field studies of the Algae Testbed Public-Private Partnership as the benchmark for algae agronomics. Sci Data 5:180267. https://doi.org/10.1038/sdata.2018.267.
- Huntley ME, Johnson ZI, Brown SL, Sills DL, Gerber L, Archibald I, Machesky SC, Granados J, Beal C, Greene CH. 2015. Demonstrated large-scale production of marine microalgae for fuels and feed. Algal Res 10:249–265. https://doi.org/10.1016/j.algal.2015.04.016.
- Xia L, Rong J, Yang H, He Q, Zhang D, Hu C. 2014. NaCl as an effective inducer for lipid accumulation in freshwater microalgae *Desmodesmus abundans*. Bioresour Technol 161:402–409. https://doi.org/10.1016/j .biortech.2014.03.063.
- Knoshaug EP, Dong T, Spiller R, Nagle N, Pienkos PT. 2018. Pretreatment and fermentation of salt-water grown algal biomass as a feedstock for biofuels and high-value biochemicals. Algal Res 36:239–248. https://doi .org/10.1016/j.algal.2018.10.024.
- Dong T, Knoshaug EP, Davis R, Laurens LML, Van Wychen S, Pienkos PT, Nagle N. 2016. Combined algal processing: a novel integrated biorefinery process to produce algal biofuels and bioproducts. Algal Res 19: 316–323. https://doi.org/10.1016/j.algal.2015.12.021.
- Laurens LML, Nagle N, Davis R, Sweeney N, Van Wychen S, Lowell A, Pienkos PT. 2015. Acid-catalyzed algal biomass pretreatment for integrated lipid and carbohydrate-based biofuels production. Green Chem 17:1145–1158. https://doi.org/10.1039/C4GC016128.
- Varela-Alvarez E, Andreakis N, Lago-Leston A, Pearson GA, Serrao EA, Procaccini G, Duarte CM, Marba N. 2006. Genomic DNA isolation from green and brown algae (*Caulerpales* and *Fucales*) for microsatellite library construction. J Phycol 42:741–745. https://doi.org/10.1111/j.1529 -8817.2006.00218.x.
- Knaus BJ, Grunwald NJ. 2018. Inferring variation in copy number using high throughput sequencing data in R. Front Genet 9:123. https://doi .org/10.3389/fgene.2018.00123.
- Carreres BM, de Jaeger L, Springer J, Barbosa MJ, Breuer G, van den End EJ, Kleinegris DMM, Schaffers I, Wolbert EJH, Zhang H, Lamers PP, Draaisma RB, Martins dos Santos VAP, Wijffels RH, Eggink G, Schaap PJ, Martens DE. 2017. Draft genome sequence of the oleaginous green alga *Tetradesmus obliquus* UTEX 393. Genome Announc 5:e01449-16. https:// doi.org/10.1128/genomeA.01449-16.
- Starkenburg SR, Polle JEW, Hovde B, Daligault HE, Davenport KW, Huang A, Neofotis P, McKie-Krisberg Z. 2017. Draft nuclear genome, complete chloroplast genome, and complete mitochondrial genome for the biofuel/bioproduct feedstock species *Scenedesmus obliquus* strain DOE0152z. Genome Announc 5:e00617-17. https://doi.org/10.1128/ genomeA.00617-17.
- 31. Wakasugi T, Nagai T, Kapoor M, Sugita M, Ito M, Ito S, Tsudzuki J, Nakashima K, Tsudzuki T, Suzuki Y, Hamada A, Ohta T, Inamura A, Yoshinaga K, Sugiura M. 1997. Complete nucleotide sequence of the chloroplast genome from the green alga *Chlorella vulgaris*: the existence of genes possibly involved in chloroplast division. Proc Natl Acad Sci U S A 94:5967–5972. https://doi.org/10.1073/pnas.94.11.5967.
- Bogen C, Al-Dilaimi A, Albersmeier A, Wichmann J, Grundmann M, Rupp O, Lauersen KJ, Blifernez-Klassen O, Kalinowski J, Goesmann A, Mussgnug JH, Kruse O. 2013. Reconstruction of the lipid metabolism for the microalga *Monoraphidium neglectum* from its genome sequence reveals characteristics suitable for biofuel production. BMC Genomics 14:926. https://doi.org/10.1186/1471-2164-14-926.
- 33. Wobbe L, Bassi R, Kruse O. 2016. Multi-level light capture control in plants and green algae. Trends Plant Sci 21:55–68. https://doi.org/10 .1016/j.tplants.2015.10.004.